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THE OESOPHAGEAL GLANDS OF URODELA.

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FOR a long time the only known instance of glands occurring in the oesophagus of an Amphibian was the familiar pepsin-producing glands of the frog's oesophagus, discovered as early as 1838 by Bischoff (1). In 1853 Leydig (8) described the occurrence of saccular glands in the oesophagus of Proteus anguineus, and, more recently, similar glands have been discovered by Kingsbury (5) in the oesophagus of Necturus maculatus.

In no other Batrachian has investigation revealed the existence of glands in the oesophagus, unless, indeed, as Klein (6) suggests, the highly branched glands found at the junction of oesophagus and stomach in Triton, and termed by Langley (7) the anterior oxyntic glands, are such.

Naturally, considerable interest has been evinced in the question of the homology of these glands one with another, and with those of the higher vertebrate classes.

In order that the problem to be solved may be clearly understood, it may be as well to recapitulate briefly the facts as they appear in the forms so far investigated.

In the frog, leaving out of consideration the pyloric glands, there are two kinds of glands occurring in the foregut. The oesophageal glands occur under a ciliated epithelium, and are large compound glands, consisting each of a number of short tubular acimi lined by pepsin-secreting cells, opening into a common duct lined by transparent mucous cells. As we pass

down the oesophagus we find that, at the point where the ciliated epithelium is succeeded by the ordinary mucigenous epithelium of the stomach, there is a gradual transition from the compound oesophageal glands to the more simple tubular glands of the stomach, the second type. The secreting cells of the two kinds of gland differ markedly from one another. Those of the gastric glands contain few zymogen granules of small size, while those of the oesophageal glands are more or less filled with very large granules, and the cells themselves are larger. Further, the oesophageal glands yield an alkaline secretion, the gastric glands an acid secretion.

In Triton, again, there are two types of gland. At the junction of oesophagus and stomach occur the anterior oxyntic glands of Langley. The difference between these glands and the other gastric glands (posterior oxyntic glands of Langley) is not so marked. The former are more highly branched and are separated from one another by a larger amount of connective tissue, but the differences in the size of the granules and in the nature of the secretion, so conspicuous in the case of the frog, are absent.

In Proteus a new structure makes its appearance in the shape of isolated sac-like glands occurring in the oesophagus. These have been fully investigated by Oppel (11), who describes them as follows : "Die Drüsen des Oesophagus haben eine rundliche Form. Sie bestehen aus einem grossen Acinus. Die Drüsen sind zusammengesetzt aus einem Ausführungsgang und dem secernierenden Theil. Ich spreche von einem Ausführungsgang, da sich die Zellen desselben von denen der Schleimhautoberfläche unterscheiden. Der Ausführungsgang besteht aus Zellen von annähernd cylindrischer Form, und zwar ist die Grenze zwischen conischem und cylindrischem Epithel stets eine scharfe. Eine besondere Eigenthümlichkeit liegt in der Uebergangsstelle von diesen cylindrischen Zellen des Ausführungsgangs zu den secernierenden Zellen. Dieselbe liegt nämlich nicht an der Stelle, an welcher die Erweiterung des engen Ganges zum Acinus stattfindet, sondern die Cylinderzellen gehen noch ein Stück weit in den Acinus hinein, um dann rasch zu den niedrigeren secernierenden Zellen abzufallen.

Diese Zellen zeigen in ihrem Protoplasma einen körnigen Bau, Körner, welche sich mit verschiedenen Farben, z. B. Eosin, S.-Fuchsin tingieren, mit Osmiumsäure bräunen . . . ." No glands resembling the anterior oxyntic glands of Triton are present in the adult, but he found in the young animal, at the junction of oesophagus and stomach, glands which are intermediate in nature between the oesophageal and gastric glands.

In Necturus there are, according to Kingsbury, three kinds of glands present. In the oesophagus are large saccular glands in most respects like those of Proteus, except that Kingsbury was unable, even after repeated trials, to demonstrate the presence of any granules capable of reducing osmic acid. At the junction of oesophagus and stomach are richly branched glands like the anterior oxyntic glands of Triton, and finally there are the ordinary gastric glands.

There are thus three types of gland occurring in the oesophagus of Batrachia, the relationship of which to one another, to the gastric glands, and to the oesophageal glands of higher vertebrates, is obscure. These are the compound pepsin-forming glands of the frog's oesophagus, the saccular glands of the oesophagus of Proteus and Necturus, and the anterior oxyntic glands of Triton and Necturus. It might be claimed for *a priori* reasons that no possible relationship could exist between the oesophageal glands and the gastric glands, but that position would necessitate a critical examination of the data on which this anatomical division of the foregut in the forms mentioned has been decided.

The writer found that Amblystoma combined, in a sense, the conditions found in Proteus and Triton, inasmuch as the glands in the larva resemble those of Proteus, the glands of the adult those of Triton. The present memoir is a brief account of the histogenesis of the glands in question.

Before passing on to a consideration of the histogenetic phenomena it is necessary to describe briefly the structure of the mucous membrane of the foregut in the adult animal.

The oesophagus is non-glandular, and is lined throughout by a ciliated epithelium, in which many goblet cells may be recognized. The ciliated epithelium is succeeded by the

ordinary cylindrical cells of the stomach at the point where the first glands appear, and the oesophagus expands suddenly into the stomach. The ciliated epithelium does not extend into the stomach.

The gastric zymogenic glands are of two kinds. The anterior oxyntic glands occupy the proximal portion of the mucous membrane and form a zone about 2 mm. in width around the oesophageal orifice of the stomach. They are much shorter than the other gastric glands and, like the corresponding glands in Triton, are more highly branched, and contain more mucous neck cells. The rest of the stomach, with the exception of the posterior third in which the pyloric glands are found, is occupied by the usual tubular glands, consisting of a body composed of granular pepsin-forming cells, a neck composed of transparent mucous cells, and a duct composed of cells resembling the surface epithelium. These glands correspond in all respects to the excellent description given of the corresponding structures in Triton by Carlier (2). To make clear the terminology employed, a bi-tubular gland is represented in Fig. 1.

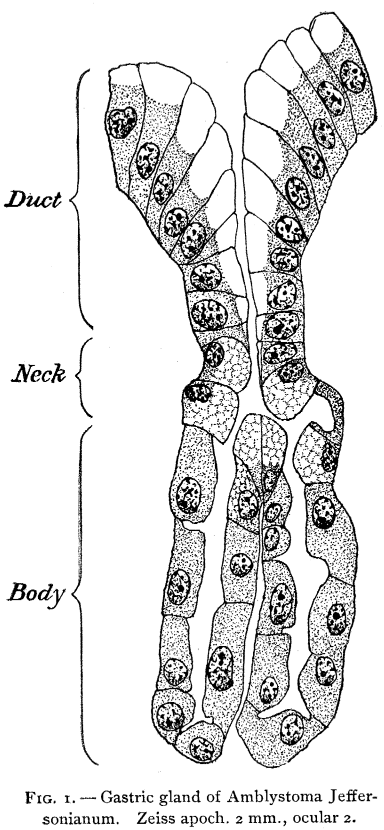

FIG. 1. — Gastric gland of Amblystoma Jeffersonianum. Zeiss apoch. 2 mm., ocular 2.

It was found necessary to resort to a new method of staining the zymogen granules, as the conventional method, by the employment of osmic acid, was not satisfactory when the cells contained brown pigment, or a great deal of prozymogen, which, as Langley (7) and Grützner (3) point out, also reduces the osmic acid, obscuring the granules if they be few in number or

of small size. For this purpose the writer employed Reinke's neutral gentian as follows: To a saturated solution of gentian violet in water a solution of orange G is added in excess. A brownish precipitate is formed which is very slightly soluble in water. This may be collected on a filter and washed until the wash water is only slightly tinged. The precipitate is then dissolved in alcohol. For use a sufficient quantity is added to twenty per cent alcohol to make a fluid of about the same color as a good solution of haemalum. Sections fastened to the slide are stained in this for twenty-four hours, all adherent stain is then removed by pressing down upon the sections several folds of filter paper, absolute alcohol added and quickly removed with the blotter, and finally oil of cloves added in which the differentiation of the stain takes place. As soon as the protoplasm of the epithelial cells appears orange, the extraction of the stain may be checked by washing in benzole, and the sections may then be mounted in the usual way in balsam. The zymogen granules are stained of an intense blue color, the nuclei blue, other portions of the cells orange. The stain is most successful after fixation in aqueous sublimate.

The earliest stages in the formation of the gastric glands are difficult to discern, owing to the great number of yolk spherules present which obscure the outlines of the cells. In a larva 11 mm. long the glands are already visible as tubular down-growths of the endoderm of the foregut. In this early larva two kinds of glands are already to be recognized, those occupying the anterior end just behind the tracheal groove, and those at the posterior end, where the stomach is as yet not clearly marked off from the general endoderm. The anterior glands are of a flask-like shape, and have a distinct lumen surrounded by a single layer of cells. Zymogen granules are not yet to be recognized, and the yolk spherules are so abundant that the outlines of the cells are not visible. In the lumen there may often be seen one or two cells, which have been, so to speak, squeezed out of the row of endoderm cells forming the gland. These cells do not take any part in the formation of the permanent histological elements, but may often be

recognized, even to a late stage of development, as disintegrating remains in these and the other gastric glands.

The posterior glands are simple tubes composed of a single layer of large yolk-filled cells surrounding a cleft-like lumen. Indeed, often it appears as if the endoderm of the foregut were in several layers without any differentiation into glands and epithelium. On careful inspection, however, it may be seen that the nuclei are arranged in an orderly fashion, as if surrounding the lumina of glands. Such an appearance is illustrated in Fig. 2. In this gland, in addition to the nuclei which are clearly arranged in a row around the lumen, two others may be seen which are nearer the center of the gland ; these are the nuclei of cells which will later be found as disintegrating remains in the lumen.

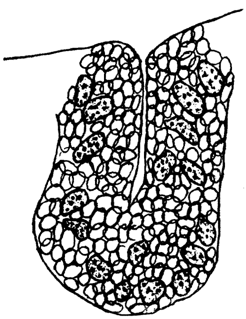

FIG. 2. — Amblystoma larva 11 mm. in length; gastric gland. Zeiss apoch. 2 mm., comp. ocular 2.

The flask-shaped glands do not, as one proceeds caudad, abruptly give place to the simple tubular glands, but there is a gradual transition.

In a larva 12 mm. in length, although the caudal portion of the stomach is still undifferentiated and the cells crowded with yolk, the yolk has sufficiently disappeared from the anterior portion to enable the shape of the glands and the cells composing them to be clearly determined. The anterior glands are now distinctly saccular, with a large lumen surrounded by a single layer of cells. The yolk spherules disappear from the cells of the glands somewhat more rapidly than from the surface epithelium, which as yet contains a considerable number. Notwithstanding the presence of the yolk, one can clearly distinguish, at this early stage, several kinds of cells, which can be readily referred to their analogues in the glands of the adult. The flask-shaped body of the gland (Fig. 3, *a*) is formed of a single layer of small cells, which vary from cubical to fusiform in shape and are usually convex towards the lumen. The protoplasm of these cells is granular and contains one or more yolk spherules. The nucleus is round or oval and rich

in chromatin. As the gland narrows into the duct (Fig. 3, *b*), these are replaced by two or three slightly larger cells, in each of which two zones may be recognized, an outer, wider, deeply staining zone containing the oval nucleus, and an inner one which stains but feebly. This inner zone exhibits a reticular structure due to the presence of a secreted substance, probably mucigen. In the more posterior tubular glands, likewise, two kinds of cells may be found, similar in all respects to those of the flask-shaped glands, granular cells occupying the body of the gland, mucigenous cells the neck. In sections stained in Reinke's neutral gentian it is found that already numerous zymogen granules are present in the deeply staining cells forming the body of the gland. In the pancreas, also, zymogen granules may be recognized long before the yolk granules have entirely disappeared from the cells.

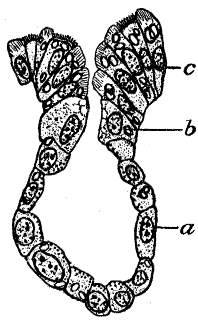

FIG. 3. — Amblystoma larva 12 mm. in length; oesophageal gland. Apoch. 2 mm., comp. ocular 2.

The epithelium of the foregut in the region occupied by the flask-shaped glands is composed of two kinds of cells (Fig. 3, *c*), ciliated cells and cells the outer ends of which stain diffusely and intensely. These obviously represent the two characteristic elements of the future oesophageal epithelium, the ciliated and the goblet cells. Over the tubular glands farther back there is only one kind of cell in the epithelium, and this is without cilia.

Mitoses may be observed with equal frequency in all the various kinds of cell composing the epithelium and glands, and all are apparently equally capable of reproduction.

The important points to be learned from this stage are that the characteristic elements of the glands are differentiated very early, that no special groups of cells have, as yet, assumed the mitotic function to the exclusion of the others, and that a portion of the glandular foregut bears a ciliated epithelium.

In a larva 14 mm. in length the foregut has advanced to a considerable degree beyond the stage last described. It is now shaped like a letter U, with a long proximal and short

distal limb, the latter curving cephalad above the ventral posterior margin of the liver, before passing into the midgut.

Four regions may now be distinguished ; a very short anterior region without glands, provided with a ciliated epithelium, a region with flask-shaped glands and ciliated epithelium, a third region with tubular or saccular glands and a mucigenous epithelium, and finally, at the posterior end, a region in which no glands at all are to be discerned. The second and third regions gradually merge into one another, but the posterior non-glandular portion is sharply marked off and forms, in part at least, the future pyloric gland region.

At this stage the two pulmonary diverticula open into a capacious pouch lying below the foregut, into the floor of which it opens. In longitudinal sections the first gland appears immediately behind this sac. Farther back more glands make their appearance, and at the point where the foregut begins to enlarge into the stomach, it is completely encircled by six or eight of these large flask-shaped glands. Farther back again the glands become less and less flask-shaped and take on a tubular or saccular character.

One of these anterior glands is represented in Fig. 4 as seen after staining in haemalum, followed by neutral gentian. The shape of the cells in the body of the gland varies with the degree of distention. There seems to be in these glands an accumulation of the secretion in the lumen distending it, for it is only by the application of a distending force from within that the extreme stretching of the cells, which may be commonly observed, could be produced. In many glands where this distention is great the cells are quite flattened and spread out over a great surface, reminding one strongly of the appearance in the mammalian blastodermic vesicle at the time of its rapid expansion. The explanation there of the flattening of the cells is clearly the stretching caused by the rapid transudation of fluid into

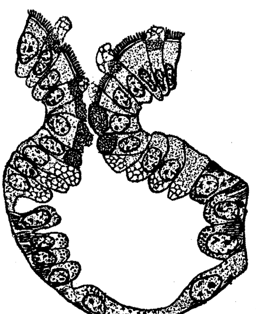

FIG. 4.—Amblystoma larva 14 mm. in length; oesophageal gland. Apoch. 2 mm., comp. ocular 2.

the vesicle, but in these glands it is difficult to explain why the fluid is not discharged into the cavity of the foregut before the pressure gets sufficiently high to cause a stretching of the cells. A possible explanation is the viscidity of the secretion owing to the large number of mucous cells in these glands.

Fig. 4 shows a gland only moderately distended, and here it is seen that the cells at the bottom, where the gland is unsupported by neighboring glands, are drawn out flat, while those at the side still retain their approximately columnar shape.

The two kinds of cells noticed in the earlier larva may still be recognized, the clear mucous cells occupying the top and neck of the flask, the granular cells the sides and base of the flask. The protoplasm of the latter now stains strongly in haematoxylin, and exhibits a faintly striated or finely vacuolated structure. This is due to the presence of prozymogen, which may also be demonstrated by the use of acid alcohol, followed by aqueous haematoxylin after the method of Macallum.[1] The inner end of the cell between the nucleus and the lumen stains but slightly in haemalum, but in sections treated with neutral gentian it is seen to be filled with perfectly round, deeply stained granules of zymogen. The neck of the flask-shaped gland is occupied by long mucous cells of a columnar shape, which also extend into the gland and form the top of the flask. In these cells two zones may be distinguished, an outer protoplasmic, which stains strongly and which contains a quantity of masked iron, and an inner transparent and reticular. The meshes of the latter are filled with a substance which stains faintly in indulin, more readily in Mayer's mucicarmine. In sections stained with neutral gentian many deeply stained granules may be seen in the mucigenous portions of these cells. These are somewhat elongated and not perfectly round, as are the zymogen granules of the other kind of cell. Their significance is not clear; it is possible that they may indicate an imperfect differentiation of the zymogenic and mucigenic functions at this stage of development.

The surface epithelium in this region of the foregut consists of alternate ciliated cells and goblet cells. Tracing the foregut

[1] *Journ. of Phys.* Vol. xxii. 1897.

backward, the glands become gradually tubular or saccular, without any appearance of distention, and the ciliated cells disappear, so that the rest of the glandular portion, as well as all the posterior non-glandular portion of the stomach, is provided with a mucigenous epithelium.

Attention should be called at this stage to the remarkable resemblance between the mucigenous border of the gastric epithelium and the cuticula of the cells in the buccal cavity. Both have a characteristic striated appearance, and one is tempted to think that they cannot be very different chemically.

The cells of the tubular glands do not differ in any respect from those of the flask-shaped glands. The mucous cells are less numerous, and a few glands may be entirely without them. The cells of all the glands, even the very last, contain both zymogen granules and prozymogen.

There are as yet no pyloric glands formed; the epithelium of the posterior portion of the stomach is perfectly smooth and without glandular outgrowths.

Even at this stage there is a remarkable resemblance between the anterior flask-shaped glands and the oesophageal glands of Proteus and Necturus, and as development proceeds this resemblance becomes more and more striking.

The mouth and pharynx are lined in the aquatic Amblystoma larva by a stratified non-ciliated epithelium, with cuticular cells and goblet cells. In a transverse series it may be seen that immediately behind the last gill slit this changes to a ciliated epithelium. One may thus consider the first ciliated cell in a longitudinal section as indicating where the oesophagus begins. Measured from this point the foregut in a larva 16 mm. long is about 3 mm. in length. Of this .49 mm. at the anterior end is non-glandular. Behind this we have a portion .45 mm. long extending from the anterior border of the first gland to a point where the foregut begins to expand to form the stomach. This would doubtless, but for the presence of glands, be regarded as a portion of the oesophagus. Beyond this again, the ciliated epithelium extends into the stomach for a distance of .35 mm. The rest of the stomach is lined by a mucigenous epithelium,

like that of the adult except in its great capacity for division. The posterior portion 1.3 mm. in length is still quite devoid of glands.

Staining with haemalum and neutral gentian shows that at this stage also the cells forming the body of every gland in the foregut contain both abundant zymogen granules and prozymogen, and it is impossible to discern any difference whatever in the cells composing the large anterior flask-shaped glands and the smaller posterior tubular glands respectively, except that in the latter there is no evidence of distention and consequent flattening.

From this stage onward the changes proceed somewhat more slowly and may be summed up briefly.

In a larva 25 mm. in length the foregut measured 5.6 mm. in length. Of this the anterior 1.4 mm. was non-glandular, showing a relatively more rapid growth in length in this portion of the foregut. The ciliated epithelium extended a further distance of .84 mm. into the stomach, the posterior portion of which, 3.36 mm. in length, was lined by the usual mucigenous epithelium.

Fig. 5 is from a larva 32 mm. in length. It is at this stage that the resemblance to the oesophageal glands of Proteus and Necturus is most marked. The duct of the gland and the portion of the wall nearest to the surface epithelium are composed of elongated cylindrical cells forming a single row. Four of these cells are represented in Fig. 6, *A*, as seen under a high magnification. Each presents an outer granular protoplasmic zone in which the oval nucleus is imbedded, and an inner more extensive zone which is coarsely reticular. They obviously represent the large, clear mucous cells of the ordinary gastric glands, and, as we shall see, are actually transformed into these in the adult. The difference in shape is dependent on external conditions, such as the grouping of the cells, and is not inherent in the cells themselves.

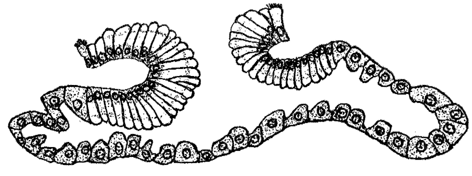

FIG. 5. — Larva of Amblystoma 32 mm. in length; oesophageal gland. Ross obj., $\frac{4}{10}$ in., Leitz ocular No. 1.

At the end of the gland they are succeeded suddenly by the zymogenic cells. In these the minute structure is obscured by the large amount of prozymogen present. The cells are less flattened than at the earlier stage of development, probably because the initial distention has been compensated by the rapid division and growth of the cells. A number of these cells is represented in Fig. 6, *B*. They are now somewhat columnar in shape, with convex ends projecting into the lumen. The nucleus is round or oval and placed in the center of the cell, though in the more columnar cell it is often nearer the lumen than the base of the cell. The free end of the cell may be seen in sections stained with neutral gentian, to be filled with granules of zymogen. Other granules may be seen at the sides of the nucleus, and a few are occasionally found in the base of the cell. The rest of the cell is occupied by a deeply staining protoplasm, which owes its ability to absorb nuclear stains to the large amount of prozymogen present, as may be shown by the employment of Macallum's methods of detecting masked iron. The distribution of the prozymogen determines the appearance of the cell, and three main types are to be recognized; in the first the stain is diffused through the whole of the protoplasm, but more pronounced at the base and sides of the cell, and on close examination a very finely vacuolated structure may be made out; in the second the whole or part of the cell exhibits long deeply staining fibrillae; and in the third type the prozymogen is distributed as small irregularly staining particles throughout the protoplasm.

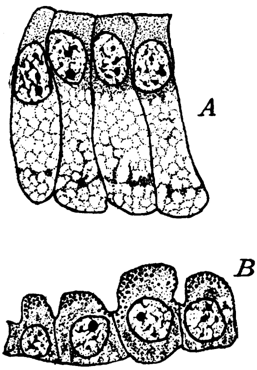

FIG. 6.—Oesophageal gland of Amblystoma larva. *A*, mucous cells; *B*, zymogenic cells. Zeiss apoch. 2 mm., comp. ocular 8.

All the three main types of cells composing the glands and surface epithelium are still capable of division, and numerous mitoses may be seen in all.

Oppel's description of the structure of the oesophageal glands of Proteus would apply word for word to these glands

of the larval Amblystoma, and in the case of Necturus I have satisfied myself, by comparison of the actual objects, that the structures are identical.

The latest larva examined was 65 mm. in length. This animal was apparently about to undergo metamorphosis, as the stratified epithelium of the mouth had been replaced by ciliated. The pyloric glands were fully developed, and the ordinary gastric glands had assumed the appearance they present in the adult. The anterior portion of the stomach, about a millimeter in extent, was still ciliated, but the saccular glands of this region had undergone considerable modification. One of these is shown in Fig. 7. It will be seen that the base of the gland has grown out into a number of short secondary tubules, formed for the most part of zymogenic cells, and the gland now consists of a large number of such tubules, each similar in structure to an ordinary gastric gland, opening into a large common cavity lined by transparent mucous cells corresponding to the neck cells of an ordinary gland. In short, the saccular gland of the embryo is being transformed into an anterior oxyntic gland of the adult.

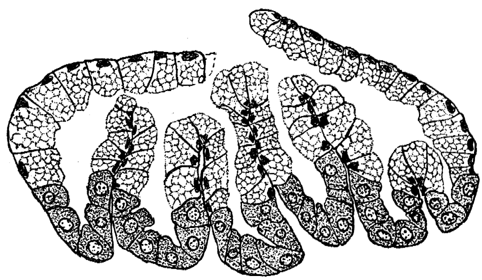

FIG. 7. — Oesophageal gland of 65 mm. larva. Ross obj. $\frac{4}{10}$ in., Leitz ocular No. 1.

Two of the most anterior glands in this larva were included in the oesophagus. All were in full physiological activity and were filled with zymogen granules.

I have been unable to secure a specimen of Amblystoma undergoing metamorphosis, or one that has just completed it, and am therefore unable to state positively whether all the saccular glands become transformed by subsequent branching into anterior oxyntic glands, or some of them degenerate and disappear. There is in the latest larva that I have examined no evidence of changes of a degenerate nature, and I am therefore inclined to believe that the most anterior glands, as well as the rest, are taken up into the stomach, and that the oesophagus

of the adult is entirely formed by rapid growth from the short non-glandular region of the early larva.

One cannot doubt that the large saccular glands of the larval Amblystoma are the homologues of the so-called oesophageal glands of Proteus and Necturus. The failure of Kingsbury, however, to detect the presence of zymogen granules in the glands of Necturus led me to reinvestigate these structures with a view of determining whether or not this was a real point of difference. At first I employed a number of specimens of Necturus which had been kept in the laboratory tank for several months without food. In these cases the results were negative, no zymogen granules were present. I afterwards obtained two specimens captured in the vicinity of Toronto, and in a perfect state of nutrition. In these no difficulty was experienced in demonstrating the presence of zymogen granules in the cells of the oesophageal glands. Kingsbury's failure is, in all probability, to be ascribed to the inadequate method he employed to demonstrate the granules. For this purpose he employed treatment of the fresh glands with osmic acid. Now it has been noted by Langley (7) and Grützner (3) that the protoplasm of ferment-secreting cells which contain a great deal of reserve material (prozymogen) stains strongly in osmic acid. For this reason its use, in cases where the granules are few in number and small, and where there is a great deal of prozymogen present, is of little value. This is precisely the condition in Necturus. By the neutral gentian method, however, the granules are stained much more strongly than the prozymogen, and no difficulty is experienced in demonstrating them when present. Fig. 8 shows a number of cells from such a preparation.

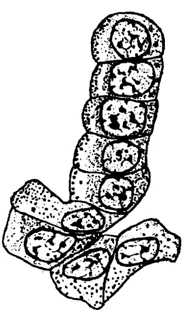

FIG. 8.—Zymogenic cells from oesophageal gland of Necturus, showing zymogen granules in free border of cells. Zeiss apoch. 2 mm., ocular 8.

It should be added, however, that the oesophageal glands of Necturus do not present the evidences of strong functional activity seen in those of the larval Amblystoma. The granules are much smaller and may be quite absent from many of the cells of the gland,

even in a well-nourished animal. This is particularly the case in those cells the inner ends of which exhibit signs of degeneration in the shape of the structures described by Kingsbury as mucous globules. It is probable that there is a tendency for these glands in Necturus to degenerate rather than remain of physiological importance.

The so-called oesophageal glands of Proteus and Necturus are really gastric glands the development of which has been arrested. There is also in these animals an arrested development of the foregut, compensation for which has been, in a measure, attained by the conversion of the anterior portion of the stomach into a functional oesophagus. Only a short anterior non-glandular portion actually corresponds to the oesophagus of other Urodela.

Two questions remain to be considered, the relation of these glands to the oesophageal glands of higher vertebrates and to the oesophageal glands of the frog.

The first question is a comparatively simple one. The oesophageal glands of higher vertebrates have no features in common with those of Batrachia and are probably of secondary origin. In Reptilia oesophageal glands are rare, and where they do occur, as, for example, in Testudo graeca, are simple crypts lined by cells similar to those of the surface epithelium, namely, ciliated cells and goblet cells, the latter predominating. In birds and mammals, where the epithelium is usually of the stratified squamous variety, they are more or less complex mucous glands. In no case, as far as I am aware, has investigation revealed in the oesophageal glands of Sauropsida or Mammalia the occurrence of ferment-secreting cells. It is probable that the oesophageal glands of higher vertebrates have arisen in response to a demand, in a very long and relatively narrow oesophageal tube, for a more efficient lubricating mechanism, and an epithelium that will withstand friction. The first step in this process is the formation of deep crypts lined by ciliated cells and many goblet cells ; the second, the disappearance of the ciliated cells from the crypts so as to form a pure mucous gland, and their replacement on the surface by a stratified squamous epithelium.

The second problem is less simple. Because of the very exceptional conditions introduced in the case of the frog by the herbivorous diet of the tadpole, and of the very extensive histolytic changes which take place in the whole intestine during metamorphosis, it becomes difficult to discuss this question from the standpoint of histogenesis. The question is, whether the oesophageal glands of the frog, like those of Proteus and Necturus, are to be regarded as somewhat modified anterior gastric glands. Let us examine, in the first place, the anatomical characters on which the subdivision of the foregut has been determined in this form. According to Wiedersheim (12) the stomach begins at a point where the foregut experiences an abrupt turn to the left. This is found on examination to correspond to the point where the ciliated epithelium is succeeded by the cylindrical epithelium of the stomach. There is also a slight constriction at this point and a change in the color of the mucous membrane. Of these the only character of importance is the change of epithelium. This is not, in my opinion, a valid criterion for the following reasons: In Amblystoma ciliated epithelium is found in the anterior portion of the stomach up to a late stage of development. In the tadpole, according to Gage, the whole foregut is ciliated, and several observers record patches of ciliated cells in the stomach of the adult frog. In several of our American "ganoids," Hopkins (4) and Macallum (9) describe the ciliated epithelium as being continued over a considerable portion of the stomach.

It is true that the differences in the cells of the oesophageal and gastric glands of the frog are very striking; but if we compare the oesophageal glands of the frog with the gastric glands of any Urodele or of Bufo, these differences are not apparent. The same cellular elements are present, with almost the same arrangement and structure.

The gastric glands of the frog are, in fact, unique among the Batrachia, in the small amount of zymogen which they contain. May this not be but another instance in which this animal, as compared with other Batrachia, exhibits an unusual degree of specialization, the anterior gastric glands (so-called oesophageal glands) having retained and developed the zymogenic

function at the expense of the oxyntic function, and the posterior the oxyntic at the expense of the zymogenic function, thus foreshadowing in a parallel way the histological differentiation which is seen in the chief and parietal cells of the gastric glands of mammals?

The conditions obtaining in the foregut of Proteus, Necturus, and the larval Amblystoma are of interest apart from their purely histological bearing. For it is obvious that, if the condition in these animals is primitive, the gastric glands of the ancestral types must have occupied a much more extensive portion of the foregut than is the case in existing forms.

Among fishes the subdivision of the foregut into oesophagus and stomach is well marked, not only among the more highly specialized Teleostomes, but also in the sharks and rays. No glands are present in the oesophagus, and the epithelium is different from that of the stomach. In Amia, Lepidosteus, and Acipenser, according to Macallum (9), it is not only extremely difficult to decide on superficial examination where the oesophagus ends and the stomach begins, but on microscopic examination the former is found to have a similar epithelium to the stomach and to contain glands. The nature of these glands is at present in doubt. No doubt the investigation of the structure and histogenesis of the elements of the foregut in these forms, and more particularly in Polypterus, will yield highly interesting and instructive results.

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